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Award Number:
W81XWH-10-1-0582

TITLE:
ETS Gene Fusions as Predictive Biomarkers of Resistance to Radiation Therapy for Prostate Cancer

PRINCIPAL INVESTIGATOR:
Felix Feng, M.D.

CONTRACTING ORGANIZATION:
The University of Michigan
Ann Arbor, MI 48109

REPORT DATE:
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Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
Felix Feng, M.D.

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5e. TASK NUMBER

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University of Michigan
Ann Arbor, MI 48109-1340

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13. SUPPLEMENTARY NOTES

14. ABSTRACT- The research goals of this grant proposal are to: 1) investigate the effect of ETS gene fusions on radiation phenotype in pre- clinical models of prostate cancer, 2) to explore the mechanism of interaction between ERG (the predominant ETS gene fusion product) and the DNA repair protein DNA-PK, and 3) to determine if ETS gene fusion status is a clinical biomarker of radioresistance for prostate cancer. The training goals of this grant proposal included a series of regular meetings with mentors, research seminars, journal clubs, and workshops, all of which are intended to help Dr. Feng develop as a transla-tional scientist. This grant proposal was approved as a five-year award; the current annual report summarizes accomplish-ments over the second year of the grant, from July 15, 2011 to July 15, 2012.Overall, the first two years of this grant have been very successful. The work accomplished as a result of this grant resulted in two publications in very high-impact journals, four national presentations, and three grants (two from the Prostate Cancer Foundation and one from Celgene). Additionally, Dr. Feng has met the training achievements specified in his original grant.The research proposed in this training grant represents an important area within the field of prostate cancer research. Because ETS gene fusions are thought to be driver alterations in over half of all prostate cancers, understanding the mecha-nistic and potential clinical implications of these gene fusions has significant ramifications, particularly in the context of radiation therapy, which represents a primary treatment modality for localized prostate cancer. In the second year of this grant period, we have generated bioluminescent ETS+ and ETS- cells in three different prostate cancer cell lines, and we havereceived institutional approval to proceed with the proposed animal studies and human biomarker tissue studies. This workbuilds on our first year findings that ERG (the predominant ETS gene fusion product) confers radioresistance in preclinical models of prostate cancer and that this radioresistance can be reversed with DNA-PK inhibition. These findings suggest that DNA-PK inhibition should be explored as a clinical strategy for radiosensitizing prostate cancers.

15. SUBJECT TERMS- Prostate cancer, ETS gene fusions, ERG, radiation resistance, DNA-PK

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a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
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Introduction

This annual report will summarize the accomplishments associated with the Department of Defense Physician Research Training Award (W81XWH-10-1-0582), awarded to Felix Feng, M.D. This award included both research goals and training goals. The research goals of this grant proposal are to: 1) investigate the effect of ETS gene fusions on radiation phenotype in preclinical models of prostate cancer, 2) to explore the mechanism of interaction between ERG (the predominant ETS gene fusion product) and the DNA repair protein DNA-PK, and 3) to determine if ETS gene fusion status is a clinical biomarker of radioresistance for prostate cancer. The training goals of this grant proposal included a series of regular meetings with mentors, research seminars, journal clubs, and workshops, all of which are intended to help Dr. Feng develop as a translational scientist, with the ultimate goals of submitting a NIH-level grant as an independent investigator and developing a translational clinical trial. This grant proposal was approved as a five-year award; the current annual report summarizes accomplishments over the second year of the grant, from July 15, 2011 to July 15, 2012.

Body

Research achievements: Tasks and Subtasks

As outlined in the original Statement of Work, this grant proposal was comprised of three specific aims, subdivided into 7 tasks, which were further divided into 20 subtasks. In year 1, I was able to complete seven subtasks (1A, 1B, 3A, 3B, 4A, 4B, and 4C), resulting in completion of Tasks #1 and #3. In year 2, I have been able to complete subtasks 2A, 2B, 6A, 7A, and 7B, resulting in progress in Tasks 2, 6, and 7. In total, I have completed 12 out of 20 proposed subtasks over the first two years of my grant, which puts me ahead of the schedule outlined in my initial statement of work (10 subtasks to be completed over the first 2 years). The findings associated with these subtasks and tasks from year 2 are detailed below.

Task #2 was to assess for the effect of ERG overexpression on radiation response in mouse xenograft experiments. Specifically, our goal was to assess the effect of radiation +/- DNAPK inhibition on ETS-positive vs ETS-negative prostate cancer xenografts. One of the barriers to achieving Task 2 has been my hesitation in proceeding with xenograft experiments with the DNA-PK inhibitor which I originally proposed to use in the grant (NU7026). Specifically, using *in vitro* clonogenic survival assays in both PC3 and DU145 prostate cancer cell lines, we have demonstrated that while knockdown of DNAPK via siRNA approaches results in significant radiosensitization (Figure 1A), the addition of the first-generation DNAPK inhibitor NU7026 to radiation results in only modest radiosensitization (Figure 1B). However, the addition of the second-generation DNAPK inhibitor NU7441 to radiation results in significant radiosensitization (Figure 1C), comparable in magnitude to siRNA knockdown of DNAPK (Figure 1A). Given the expense and time associated with large mouse xenograft experiments, I would prefer to proceed with the strongest DNAPK inhibitor possible for *in vivo* studies. In fact, I have recently received grant funding from the pharmaceutical company Celgene to assess the effect of their clinical-grade DNAPK inhibitor CC115 (which is currently in phase I trials) on ETS-positive versus ETS-negative prostate cancer xenografts. This Celgene grant does not overlap with my DOD grant because it does not involve radiation--though I am currently trying to convince the Celgene company to allow me to use their CC115 drug in radiation-based xenograft experiments.

Due to the issues highlighted above, I have been uncertain of how to proceed with the xenograft studies. On one hand, I can proceed with the combination of radiation and the first-generation DNAPK inhibitor NU7026, as originally proposed in my application (as this was the only DNAPK

inhibitor available when I submitted my application). A second option would be to proceed with the combination of radiation and the second-generation DNAPK inhibitor NU7441, which would require an amendment to my DOD grant. A third option would be to proceed with the combination of radiation and the third-generation DNAPK inhibitor CC115, which would require both an amendment to my DOD grant as well as obtaining permission from Celgene to use their drug in combination with radiation. I plan on discussing with my DOD scientific review officer which of these three options to proceed with. However, during the past year, to prepare my group to proceed with any of these three options, I have submitted an institutional animal use application (UCUCA) which allows me to combine any of these three agents with radiation for xenograft studies. This application was approved by my institutional University Committee on Use and Care of Animals in March 12 (UCUCA protocol: Appendix 1; approval letter for protocol: Appendix 2). This approval represents the completion of subtask 2A. To also prepare for proceeding with the xenograft experiments, I have created bioluminescent cells from three tumorigenic cell lines (DU145, PC3, and VCaP) and their corresponding clones of ERG knockdown or overexpression, via transduction of a pLentilox-CMV-Luciferase vector, resulting in completion of subtask 2B.

Tasks #6 and #7 comprised Specific Aim 3, which was to evaluate whether ETS gene fusion status is a predictive biomarker of resistance to radiation therapy in prostate cancer patients treated with external beam radiation. Specifically, Task #6 was to determine ETS gene fusion status in prostate cancer specimens from patients treated with radiation, and Task #7 was to determine the association between fusion status and clinical outcomes. My initial plan was to use a previously assembled institutional prostate cancer tissue set comprised of 281 specimens from men treated with radiation for prostate cancer. However, while trying to perform a separate (unrelated to this grant) biomarker study on these specimens, my pathologist (Rohit Mehra) and I realized that, unfortunately, the majority of these specimens are exhausted, from the perspective of cancer-containing tissue. More specifically, while the core biopsies from these specimens still exist, most of the cancer-containing tissue in these core biopsies had been exhausted in previous biomarker studies. Unfortunately, we could not determine this until we actually cut into each biopsy specimen and analyzed the biopsy slices under the microscope.

In order to continue with Specific Aim 3, I obtained another source of tumor specimens from men treated with radiation therapy for prostate cancer. I applied for tissue specimens from the phase III RTOG 96-01, which was run by the Radiation Therapy Oncology Group (RTOG), a national clinical trials cooperative group. RTOG 96-01 randomized 771 patients with PSA recurrences following prostatectomy to radiation therapy alone versus radiation therapy combined with androgen deprivation therapy. Of the 771 patients enrolled on the trial, prostate cancer samples are available for 88% of them, and the current median follow-up for these patients is 9.9 years. This cohort is exceptional in that it represents a large patient population with aggressive prostate cancer treated with radiation, with long-term clinical outcomes. The addition of androgen deprivation therapy to radiation resulted in a 17% decrease in recurrence rates (from 60% for the radiation alone group down to 43% for the combination therapy group), as well as a 6% decrease in the distant metastasis rate (from 13% in the radiation alone group to 7% in the combination therapy group). Both of these decreases were statistically significant, as reported in abstract form at the 2010 ASTRO Annual Meeting[1].

After a length application and review process, the RTOG steering committee approved my request to assess ETS fusion status in these tissues. Their approval letter is included as Appendix 3. Because all these patients were prospectively consented for general biomarker studies on their tissues, the approval by RTOG for these tissues carries approval from the IRB of the American College of Radiology (which oversees RTOG). Thus, I have accomplished

subtask 6A, which is to obtain IRB approval to assess ETS gene fusion status in prostate cancer specimens. In addition, since these patients prospectively consented to have their clinical outcomes followed as part of the clinical trial, I also have IRB approval to review clinical outcomes from these patients (subtask 7A), and have access to their clinical data (which was to be compiled in subtask 7B). Overall, because I will now be examining ETS fusion status in prostate cancer patients treated with radiation on a prospective multi-institutional clinical trial (instead of retrospective single-institution cohorts), my biomarker studies (Tasks 6 and 7) will now be much stronger. I have not proceeded with any of the biomarker studies per se, as I plan on first communicating with the DOD IRB office to determine what appropriate documentation needs to be sent to the DOD before I can proceed with the biomarker research.

Thus, to summarize, Year 2 of my grant period has been marked with the identification of potential barriers to my original intended plan--specifically, with the recognition that the DNAPK inhibitor that I had originally planned on using for xenograft studies (NU7026), was ineffective in *in vitro* assays, and with the realization that the tissue samples that I had planned on using for human biomarker studies was exhausted in terms of the cancer-containing component. However, I have developed solutions to overcome these barriers (using a stronger next-generation DNAPK inhibitor instead of NU7026, and obtaining access to a better tissue bank than the one I originally specified), and have accomplished 5 subtasks in line with these solutions.

Research achievements: Milestones

In the original Statement of Work, 11 milestones were identified, and targeted over the 5 year course of this grant. In year 1, I was able to complete Milestones #2, #4, and #5, for a total of 3 out of 11 milestones reached. In year 2, based on the work above, I was able to complete Milestones 1, 7, and 8, for an additional 3 milestones, and a total of 6 out of 11 milestones reached during the first 2 years of this proposal. Specifically, Milestone #1 included institutional approval of my animal protocol, which is detailed in the above section. Milestone #7 consisted of obtaining IRB approval for analysis of the tissue bank cohort, and Milestone #8 consisted of annotating the clinical data for the tissue bank cohort, both of which have been accomplished with a switch to the RTOG tissue bank, as detailed above.

Training achievements

In my original grant application, I highlighted a series of training program activities which I hoped would contribute substantially to my scientific development. Over the past year, as proposed, I have continued to attend a number of basic science seminars, hosted by the Departments Medicine, and Molecular and Cellular Biology, which have broadened by scientific knowledge within my field. I have also regularly attended Gene Fusion and Cancer Biology Research Meetings, run by my mentor Arul Chinnaiyan, as well as the Pathology and Radiation Oncology Research Seminars, run by the two departments with which I am affiliated. Additionally, I have renewed my “Training in the Responsible Conduct of Research” certification, and presented at the national meetings noted above in the milestones section. Finally, I have met regularly with my mentors, Drs. Arul Chinnaiyan, Ted Lawrence, and Tom Carey, as planned in my original proposal.

Career achievements

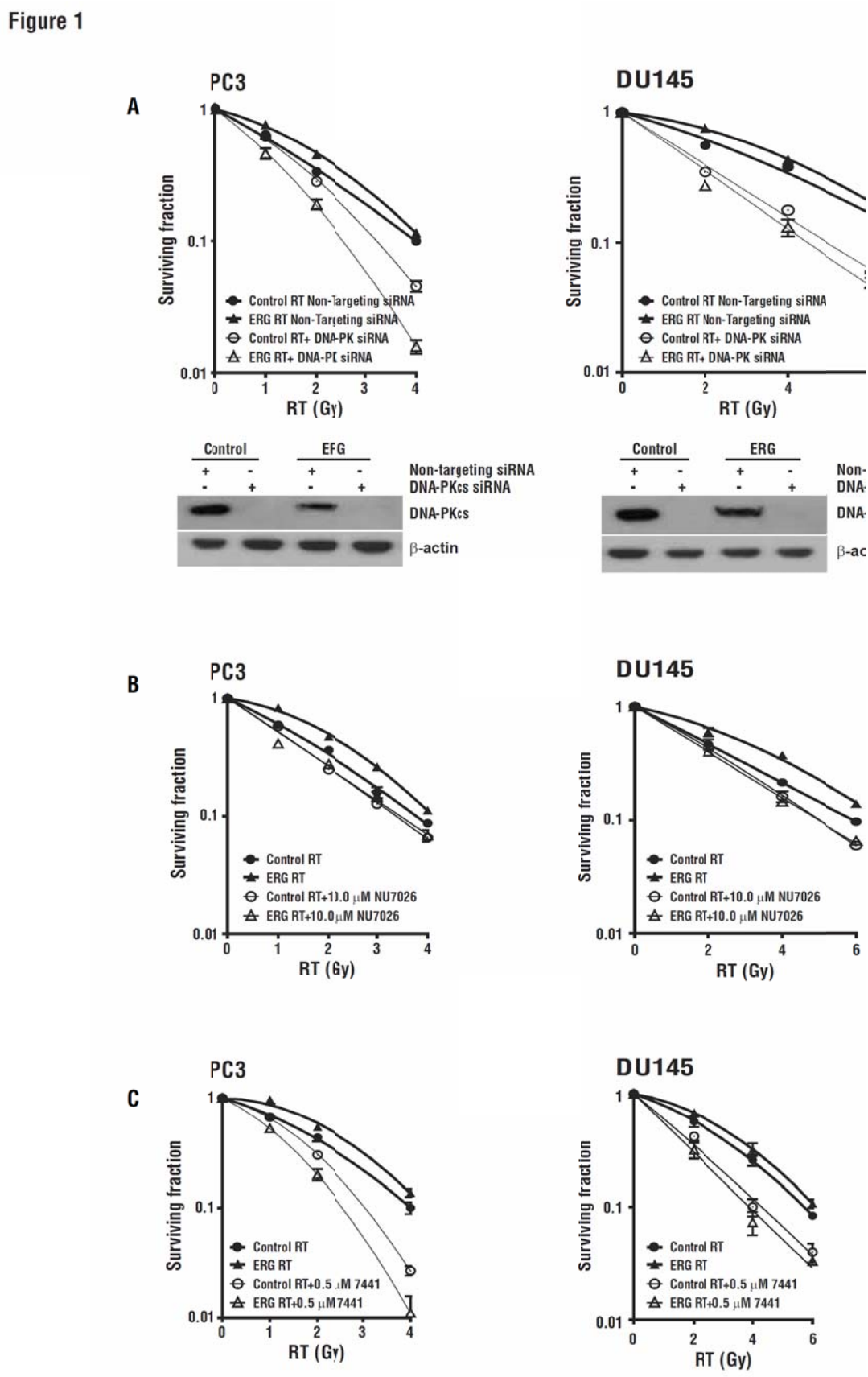
The overall goal of my DOD Mentored Physician Research Training Award was to help me develop a career as a physician scientist committed to prostate cancer research. The first two years of this award have really helped launch my career in this regard. Because of my need to obtain tissue specimens to fulfill Aim 3 of this grant, I approached the Radiation Therapy Oncology Group (RTOG), and began regularly attending their Genitourinary Cancer

Translational Research Committee meetings. Because of my increasing involvement with this group, I was appointed as chair of this committee over the past year. As chair of this committee, my role is to help direct RTOG-based prostate cancer research on a national level. This role has resulted in national recognition, as I was asked to present my research from this DOD grant in last year's AACR Prostate Cancer conference. Similarly, I have accepted an invitation to moderate one of the 3 sessions at this upcoming year's ASCO GU conference (my session will be focused on translational research in prostate cancer). Over the past year, I have also served as a grant reviewer for the NIH Cancer Biomarker Study Section (twice), a DOD study section (the Experimental Therapeutics section for Postdoctoral grants and Physician Research Training Awards), and several Prostate Cancer Foundation grant review boards. My DOD-sponsored project has led to the preliminary data necessary for several grants that I have received over the past two years, including a recent Celgene Translational Award ($500,000 over 2 years) and a Prostate Cancer Challenge Award ($1,000,000 split among 4 co-Principal Investigators over 2 years). In addition, I have submitted applications as co-PI for several DOD grants in the current funding cycle, and plan on submitting my first R01 this upcoming year. I would like to thank the DOD for making all of this possible for me.

Figures:

Figure 1: ERG causes radiation resistance, which is reversed by DNA-PK inhibition or knockdown: In both PC3 and DU145 cells, ERG overexpression resulted in a 1.3 fold increase in clonogenic survival following radiation (shown in the shift from the heavy black line with circles to the heavy black line with triangles). DNAPK knockdown or inhibition was achieved with siRNA approaches (Figure 1A), the DNAPK inhibitor NU7026 (Figure 1B), or the DNAPK inhibitor NU7441 (Figure 1C). Each of these preferentially radiosensitized ERG-positive cells compared to ERG-negative cells, with an enhancement ratio of 1.6-1.7 (for the siRNA and NU7441 approaches, Figures 1A and 1C) or an enhancement ratio of 1.2 (for the NU7026 approach, Figure 1B). Western blots in Figure 1A confirm genetic knockdown of DNAPK with siRNA approaches. Drug concentrations of DNAPK inhibition were selected based on previously reported results in the public literature, for maximum *in vitro* doses based on predicted attainable serum concentrations. As one can see, at the published maximum doses, the first-generation DNAPK inhibitor NU7026 is significantly less potent as a radiosensitizer compared to the second-generation DNAPK inhibitor NU7441.

Figure 1


A
PC3
DU145
Surviving fraction
RT (Gy)
Control RT Non-Targeting siRNA
ERG RT Non-Targeting siRNA
Control RT+ DNA-PK siRNA
ERG RT+ DNA-PK siRNA
Control
ERG
Non-targeting siRNA
DNA-PKcs siRNA
DNA-PKcs
β-actin
B
PC3
DU145
Control RT
ERG RT
Control RT+10.0 μM NU7026
ERG RT+10.0 μM NU7026
C
PC3
DU145
Control RT
ERG RT
Control RT+0.5 μM 7441
ERG RT+0.5 μM 7441

Key Research Accomplishments:

The key research accomplishments from the second year of this grant proposal include the following:

- Generation of 3 different pairs of bioluminescent ETS+ and ETS- prostate cancer cell lines, for use in my proposed xenograft studies
- Submitting an animal use protocol and receiving institutional UCUCA approval for my proposed xenograft studies (Appendices 1 and 2)
- Approval of my application for prostate cancer tissues from the national phase III RTOG 96-01 trial, for use in my proposed biomarker studies (Appendix 3)

These accomplishments add to the findings from the first year of the grant proposal, which showed that:

- ERG overexpression in prostate cancer cell lines confers radiation resistance
- This ERG-associated radiation resistance is mediated by increased efficiency of DNA repair in response to radiation
- ERG interacts with the repair protein DNAPK in a DNA-independent manner, at its tyrosine 373 site
- DNAPK knockdown or inhibition preferentially radiosensitizes ERG-positive vs ERG-negative cells, and can reverse ERG-mediated radiation resistance

Reportable Outcomes:

The second year of work from this grant proposal has resulted in the following reportable outcomes:

1) Oral presentation of work from this grant proposal, at the 2012 AACR Prostate Cancer Conference
2) Invited review (on targeting ETS gene fusions), which has been submitted to the journal *Clinical Cancer Research* (and is under review)
3) A funded Challenge Grant from the Prostate Cancer Foundation ($1,000,000 over 2 years, split among 4 co-principal investigators, of which I am one), entitled “Interrogating DNA repair aberrations in advanced prostate cancer”
4) A funded Translational Award from the pharmaceutical company Celgene ($500,000 over 2 years, on which I am PI), entitled “CC115 as a therapeutic approach for metastatic Ewing’s sarcoma or prostate cancer”

These outcomes add to the following reportable outcomes from the first year of the grant:

5) Publication of work from Task #4 in a *Cancer Cell* manuscript[3], co-published with my mentor and primary collaborator, Dr. Arul Chinnaiyan[2]
6) Oral presentation on work from Task #4, at the 2010 American Society of Therapeutic Radiology and Oncology Annual Meeting[3]
7) Poster discussion presenting work from Tasks #1 and #3, at the 2011 American Society of Clinical Oncology Annual Meeting[4]
8) Invited oral presentation on work from Tasks #1 and #3, at the 2011 Prostate Cancer Foundation Annual Meeting
9) A funded Young Investigator Award from the Prostate Cancer Foundation ($225,000 over 3 years), entitled “*Cooperativity between TMPRSS2:ERG Gene Fusions and PTEN Genomic Deletions in the Radiation Resistance of Prostate Cancer”,* from January 2011 to January 2014

Conclusion:

This Annual Report summarizes the second-year accomplishments associated with the Department of Defense Physician Research Training Award (W81XWH-10-1-0582), awarded to

Felix Feng, M.D. Overall, the second year of this grant period has been successful, and has resulted in one submitted publication (*Clinical Cancer Research)*, two funded grants, one presentation, and a national leadership position. These accomplishments add to those achieved during the first year of this grant, including one publication (*Cancer Cell)*, one funded grant, and three presentations. In total, the first two years of this grant have resulted in three subsequent funded grants, two high-tier publications, four presentations, and a national leadership position. In addition, I have completed 12 out of the 20 subtasks proposed for this 5 year grant, and am ahead of the schedule proposed in the initial Statement of Work, despite having to overcome unexpected barriers to success in both the proposed xenograft and biomarker work. Additionally, I have met the training achievements specified in my original grant.

The research proposed in this training grant represents an important area within the field of prostate cancer research. Because ETS gene fusions are thought to be driver alterations in over half of all prostate cancers, understanding the mechanistic and potential clinical implications of these gene fusions has significant ramifications, particularly in the context of radiation therapy, which represents one of the primary treatment modalities for localized prostate cancer. Our first-year findings are that ERG confers radiation resistance in preclinical models of prostate cancer and that this radiation resistance can be reversed with DNAPK inhibition. These findings suggest that DNA-PK inhibition should be explored as a clinical strategy for radiosensitizing prostate cancers. In addition, our second year accomplishments have now paved the way for us to continue with the necessary xenograft and human biomarker studies necessary to translate this work to the clinic.

I would like to thank the DOD review committee for providing me this grant to accomplish the proposed research.

References:

1) Shipley W, Hunt D, Lukka H, et al: Report of RTOG 9601: a Phase III Trial in Prostate Cancer: Anti-androgen Therapy (AAT) with Bicalutamide during and after Radiation Therapy (RT) Improves Freedom from Progression and Reduces the Incidence of Metastatic Disease in Patients following Radical Prostatectomy (RP) with pT2-3, N0 Disease, and Elevated PSA Levels. International Journal of Radiation Oncology, Biology, Physics. 78:S27, 2010
2) Brenner JC, Ateeq B, Li Y, et al: Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer. Cancer Cell 19:664-78, 2011
3) Sabolch A, Brenner JC, Ateeq B, et al: Targeted Radiosensitization of ETS Gene Fusion- Positive Prostate Cancer. International Journal of Radiation Oncology, Biology, and Physics 78:S113, 2010
4) Feng FY, Han S, Brenner C, et al: PARP inhibition reverses radiation resistance conferred by ETS fusions in prostate cancer. J Clin Oncol 29:4545, 2011

Appendices:

Appendix #1: UCUCA protocol which includes the proposed xenograft studies in this application (combining DNAPK inhibition with radiation for the treatment of prostate cancer xenografts).

Appendix #2: Approval letter for institutional UCUCA board for the above protocol (in Appendix 1)

Appendix #3: Approval letter from the steering committee of the Radiation Therapy Oncology Group allowing for use of the tissues from RTOG 96-01 for biomarker evaluation for ETS gene fusion status.

PI: Feng, Felix Y
ID: PRO00003667
Status: Protocol Approved
Expiration Date: 3/19/2015

General Information

Protocol Title:
DNAPK inhibition as a strategy for targeting ETS fusions in prostate cancer, T-lineage acute lymphoblastic leukemia, and ewing's sarcoma

Explain in non-scientific terms the long-term or overall scientific goals of the proposed work (the ultimate question this project will address).
The long-term scientific goals of this proposed work is to determine whether DNAPK inhibitors can be used, alone or in combination with cytotoxic drugs or radiation, to preferentially target prostate cancer, T-lineage acute lymphoblastic leukemia, or Ewing's sarcoma cancers. This proposed work should provide the necessary data in mouse models to support the development of a clinical trial, in patients, using DNAPK inhibitors in this context.

Explain in non-scientific terms the long-term or overall scientific objectives of the proposed work, should be specific to the proposed work.
The long-term scientific goals of this proposed work is to determine whether DNAPK inhibitors can be used, alone or in combination with cytotoxic drugs or radiation, to preferentially target prostate cancer, T-lineage acute lymphoblastic leukemia, or ewing's sarcoma tumor xenografts which harbor ETS gene fusions.

Explain in non-scientific terms the ways the proposed animal use might benefit human or animal health, the advancement of knowledge, or the good of society.
Patients with advanced Ewing's sarcomas, T-lineage acute lymphoblastic leukemia, or aggressive prostate cancers have poor outcomes. Better approaches of targeting these cancers are necessary. Almost all Ewing's sarcomas, T-lineage acute lymphpoblastic leukemia, as well as half of prostate cancers, harbor ETS gene fusions, which are abnormal genetic events which result in aggressive malignant features. The goal of this study is to explore the use of DNAPK inhibitors (a class of drugs) as an approach to selectively inhibiting tumor growth in patients with ETS-positive disease. This will hopefully improve outcomes in patients with these aggressive diseases.

Explain why animals must be used to accomplish your proposed work.
Compared to cell line experiments, tumor growth and treatment in animals, such as mice, are believed to better represent cancer growth and therapy in human beings. Because our eventual goal is to move our studies to clinical trials in humans, we want the most relevant and representative preclinical data in animals prior to assessing these therapies in humans.

Indicate the types of animal use proposed in the application. (Check all that apply)

☑ **Basic / Applied Research**

☑

PI: Feng, Felix Y
ID: PRO00003667
Status: Protocol Approved
Expiration Date: 3/19/2015

Field Research

Instruction or Training

☑ **Service (breeding, core facility)**

☑

Stem Cell Research

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PI: Feng, Felix Y
ID: PRO00003667
Status: Protocol Approved
Expiration Date: 3/19/2015

Sponsor / Funding Information

Will this project be sponsored externally? ◉ ◉ **Yes** ○ ○ No

Will this project be sponsored internally? ○ ○ Yes ◉ ◉ **No**

External Sponsor / Funding

Sponsor	PAF ID	PAF Title	Contact PI	State	Vertebrate Animals?	Current as of	Attached Document
NIH-DHHS-	07-4897	SPORE in Prostate	Kenneth	Active	yes	9/6/2012	no

PI: Feng, Felix Y
ID: PRO00003667
Status: Protocol Approved
Expiration Date: 3/19/2015

US		Cancer	Pienta			12:21 PM	
	09-PAF03054	ETS Gene Fusions as Predictive Biomarkers of Resistance to Radiation Therapy For Prostate Cancer	Felix Feng	Active	yes	9/6/2012 12:21 PM	no
Celgene Corporation	12-PAF04484	Investigating CC-115 as a Therapeutic Approach for Metastatic Ewing's Sarcoma and Prostate Cancer	Felix Feng	Active	yes	9/6/2012 12:21 PM	no

Project Title	Dept/Unit	Peer Review	Peer Review Source	Attached Documents?

There are no items to display

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PAF Number	Sponsor	PAF Title	Contact PI	State	Current of
07-4897	Health and Human Services, Department of-National Institutes of Health	SPORE in Prostate Cancer	Kenneth Pienta	Active	2/6/201

External Sponsor/Funding Documents

Title	Version Number

Select Document

External Sponsor / Funding

PAF	Sponsor	PAF Title	Contact PI	State	Current

PI: Feng, Felix Y
ID: PRO00003667
Status: Protocol Approved
Expiration Date: 3/19/2015

Number					of
09-PAF03054	Select Sponsor	ETS Gene Fusions as Predictive Biomarkers of Resistance to Radiation Therapy For Prostate Cancer	Felix Feng	Active	2/6/2012

External Sponsor/Funding Documents

Title	Version Number
Select Document	

External Sponsor / Funding

PAF Number	Sponsor	PAF Title	Contact PI	State	Current of
12-PAF04484	Celgene Corporation	Investigating CC-115 as a Therapeutic Approach for Metastatic Ewing's Sarcoma and Prostate Cancer	Felix Feng	Active	

External Sponsor/Funding Documents

Title	Version Number
Select Document	

PI: Feng, Felix Y
ID: PRO00003667
Status: Protocol Approved
Expiration Date: 3/19/2015

Personnel

Name	Protocol Role(s)	Contact Information	Alt Contact Information

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Wei Chen

Use Current UM employee contact information? **Yes** No

Description of experience and qualifications for handling animals:

Dr. Chen has a Ph.D. and has previously worked with mice in his former lab in China. Prior to beginning his experiments on this project, he will take the appropriate animal care courses required by the University of Michigan.

Role Name
Animal Handler

Felix Feng

Use Current UM employee contact information? **Yes** No

Description of experience and qualifications for handling animals:
M.D. More than 4 years experience in mouse experiments, including several projects including mouse tumor xenografts. Have been working on radiation studies in mice for the entire 4 year period.

Role Name
Principal Investigator
Lab Contact/Email Recipient
Authorized Signer
Animal Health Contact
Application/Protocol Editor

Sumin Han

Use Current UM employee contact information? **Yes** No

Description of experience and qualifications for handling animals:
Ph.D She has received all the training courses required by ULAM for handling mice.

Role Name
Animal Handler

Meilan Liu

Use Current UM employee contact information? **Yes** No

Description of experience and qualifications for handling animals:

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BS Meilan has more than 4 years experience working with mice at UM. She is experienced in oral gavage, i.v. & i.p. dosing, tumor measurement. She has had least one year experience handling rats doing blood collection at UCLA.

Role Name
Animal Handler

Kari Wilder-Romans

Use Current UM employee contact information? **Yes** No

Description of experience and qualifications for handling animals:
B.A., MPH. Research Technician, 3 years handling animals.

Role Name
Lab Contact/Email Recipient
Authorized Signer
Animal Handler
Animal Health Contact
Application/Protocol Editor

Yu Zhang

Use Current UM employee contact information? **Yes** No

Role Name
There are no items to display

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Species Information

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Species	Animals Acquired	Transportation	Quarantine / Conditioning Information
Mouse	ULAM: Yes Non-ULAM: No	Follow Guidelines: Yes Personal Vehicle Use: No	*Not Applicable For This Species*

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Mouse

Justify selected species.

This study will utilize immunocompromised mice because they are capable of accepting xenografts. Mice are the smallest animals which can be used for these studies. Also, there is ample data on the response of mice with xenografts to radiation and to the proposed drug (used individually). This minimizes the number of animals required for preliminary testing.

Non-ULAM Source Acquired:	no
Environmental Enrichment Provided:	
Animal Group Housed:	
Follow ULAM Quarantine Protocol:	
Personal Vehicle Use:	no
Follow Transportation Guidelines:	yes

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Location Information

Mouse

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Mouse

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Location	Purpose Information	Responsible Person
MED SCI I - 4310	Housing: No Use: Yes	*Not Applicable*
MED SCI I - 4433	Housing: No Use: Yes	*Not Applicable*
MSRB I - 2512B	Housing: Yes Use: Yes	*Not Applicable*

Select Location

MED SCI I - 4310

Indicate the intended purpose(s) for this location by answering the questions below.

Housing? Yes **No**

Use? **Yes** No

Will recovery surgery be conducted in this location? Yes **No**

Will non-recovery surgery be conducted in this location? Yes **No**

Select who will be responsible for meeting Animal Care Standards at the University of Michigan.

Select Location

MED SCI I - 4433

Indicate the intended purpose(s) for this location by answering the questions below.

Housing? Yes **No**

Use? **Yes** No

Will recovery surgery be conducted in this location? Yes **No**

Will non-recovery surgery be conducted in this location? Yes **No**

Select who will be responsible for meeting Animal Care Standards at the University of Michigan.

Select Location

MSRB I - 2512B

Indicate the intended purpose(s) for this location by answering the questions below.

Housing? **Yes** No

Use? **Yes** No

Will recovery surgery be conducted in this location? Yes **No**

Will non-recovery surgery be conducted in this location? Yes **No**

Will ULAM provide daily care? **Yes** No

Select who will be responsible for meeting Animal Care Standards at the University of Michigan.

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Procedures

Protocol Procedures:

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Procedure
Anesthetic, Analgesic, Tranquilizing or Neuromuscular Blocking Agents
Blood Collection
Cardiac Puncture
Euthanasia
Fee for Service
Gavage
Hazardous Agents (Including Human Tissue/Fluid)
Injection
Irradiation
Tumor Growth, Experimentally Induced

Working with the Procedures page

Select the procedures for each species.

Save the page.
Some procedures require additional information (i.e., procedure details).

Click the edit button to open the details page for the corresponding procedure.
All procedure details must be complete prior to submission.
Indicates that a procedure is complete for selected species (including procedure details if applicable).
Indicates that procedure details are not complete for selected species.
Indicates that there are procedure details for a selected procedure and can be accessed by clicking the icon.

Procedure
Addiction or Addiction Withdrawal
Adoption of Animals
Amputation
Anesthetic, Analgesic, Tranquilizing or Neuromuscular Blocking Agents

Antibody Production

Ascites Production

Behavior Modification/Operant Conditioning

Blood Collection

Breeding (Mating/Parturition)

Burn

Cannulation/Catheterization

Capture of Wildlife

Cardiac Puncture

Environmental Manipulation

Euthanasia

Fee for Service

Food/Water Manipulation

Food/Water Restriction

Gavage

Hazardous Agents (Including Human Tissue/Fluid)

Hypo/Hyperthermia

Imaging

Immunization, Experimental

Immunosuppression

Implant

Inflammation, Experimentally Induced

Injection

Injury/Trauma

Inoculation, Experimental

Irradiation

Noxious Stimulus

Obesity, Experimentally Induced

Organ/System Failure/Dysfunction, Experimentally Induced

Paralysis, Experimentally Induced

Performance Test

Predator/Prey Animal

Restraint, Prolonged (30 minutes or longer)

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Sepsis Induction/Infection
Stress, Experimentally Induced
Surgery
Tether
Tissue Collection for Genotyping
Toxicity Test
Tumor Growth, Experimentally Induced
ULAM Technical Services

Adverse Consequences	Alternatives Required	Animal Form Used	Animal Group Housed	Animal Handling Details	dateCreated	dateModified	Environmental Enrichment Provided	Follow Transportation Guidelines	Follow ULAM Quarantine Protocol	ID
Adverse Consequences	false				2/6/2012	3/6/2012		yes		ID00162029

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Mouse

Procedure Details - Anesthetic, Analgesic, Tranquilizing or Neuromuscular Blocking Agents

Immunizing Agent	Type	Adjuvant
There are no items to display		

Will ULAM Breeding Colony Management services be used? ○ Yes ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ Yes ○ No

Fee for Service Protocol
There are no items to display

Restraint/Device
There are no items to display

Tissue Type

Ear

Tail

Toe

Other

Agent
Isoflurane - Vaporizer

Anesthetic/Analgesic Details**Follow Anesthesia and Analgesic Guidelines:** **Yes** No
Guideline Deviation Description:

Neuromuscular Blocking Agent Details **Neuromuscular Blocker Justification:**

Neuromuscular Blocker Pain Awareness Description:

Agent	Type	Dose	Unit
Isoflurane - Vaporizer	Anesthetic	4 -- 5	%

Does the Dose Range differ from the recommendation? Yes **No**

Route of Administration (select all that apply)

Route

Inhalation

Does the Route of Administration differ from the recommendations? ○ ○ Yes ◉ ◉ **No**

Duration of Anesthesia	**Unit**	**Max Supplemental Dose (See Guidelines)**	**Unit**
5-10	Minute(s)	1-2	%

Select method(s) for scavenging waste gases.

Filtering with a charcoal canister

Will there be variations from standard anesthetic administration? ○ ○ Yes ◉ ◉ **No**

Is there any additional information related to this agent? ○ ○ Yes ◉ ◉ **No**

Immunizing Agent	**Type**	**Adjuvant**
There are no items to display		

Mouse

Procedure Details - Blood Collection

Will ULAM Breeding Colony Management services be used? ○ ○ Yes ○ ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ Yes ○ No

Fee for Service Protocol

There are no items to display

Restraint/Device

There are no items to display

Tissue Type
Ear
Tail
Toe
Other

Method
Saphenous vein
Lateral tail vein

Will the ULAM Guidelines for Blood Collection from Laboratory Animals be followed? ◉ **Yes** ○

Immunizing Agent	Type	Adjuvant

There are no items to display

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Will ULAM Breeding Colony Management services be used? ○ Yes ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ Yes ○ No

Fee for Service Protocol
There are no items to display

Restraint/Device
There are no items to display

Tissue Type
Ear
Tail
Toe
Other

Immunizing Agent	Type	Adjuvant

There are no items to display

Will ULAM Breeding Colony Management services be used? ○ Yes ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ Yes ○ No

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Mouse

Procedure Details - Euthanasia

Fee for Service Protocol
There are no items to display

Restraint/Device
There are no items to display

Tissue Type
Ear
Tail
Toe
Other

Euthanasia Method
Carbon dioxide overdose

Methods used to ensure that the animals will not revive. Select all that apply.

☑	**Induction of Bilateral Pneumothorax**
☑	
☑	**Other**
☑	
	Decapitation

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☑ **Removal of Vital Organ**

☑

Other Description:
exanguination

Will animals be perfused with paraformaldehyde or another fixative? ○ ○ Yes ◉ ◉ **No**

Euthanasia Method: Carbon dioxide overdose **Classification:** Acceptable

Agent
Carbon Dioxide (CO2)

Agent
Carbon Dioxide (CO2)

Route of Administration (select all that apply)

Route
Inhalation

Immunizing Agent	Type	Adjuvant
There are no items to display		

Will ULAM Breeding Colony Management services be used? ○ ○ Yes ○ ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ ○ Yes ○ ○ No

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Mouse

Procedure Details - Fee for Service

Fee for Service Protocol
ULAM Breeding Colony Management and Technical Services

Restraint/Device
There are no items to display

Tissue Type
Ear
Tail
Toe
Other

Fee for Service Protocol	Describe the procedures conducted under this Fee for Se
ULAM Breeding Colony Management and Technical Services	We will utilize the University of Michigan Breeding Colony to ma

Immunizing Agent	Type	Adjuvant
There are no items to display		

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Will ULAM Breeding Colony Management services be used? ○ Yes ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ Yes ○ No

Fee for Service Protocol
There are no items to display

Restraint/Device
There are no items to display

Tissue Type
Ear
Tail
Toe
Other

Immunizing Agent	Type	Adjuvant

There are no items to display

Will ULAM Breeding Colony Management services be used? ○ Yes ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ Yes ○ No

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Fee for Service Protocol

There are no items to display

Mouse

Procedure Details - Hazardous Agents (Including Human Tissue/Fluid)

Restraint/Device

There are no items to display

Tissue Type
Ear
Tail
Toe
Other

Will ULAM Animal Containment Policies and Procedures be followed? **Yes** No

Substance/Agent

Substance/Agent	Recombinant DNA
Immortalized Human Tumor Cell Lines Amount: 1-10 million Cells Hazard Type: Biological Animals per administration: 1004 ROA: IV, SC	Recombinant DNA:
Olaparib Amount: 100 mg/kg Hazard Type: Chemical Animals per administration: 50 ROA: IP	

NU7026
Amount: 25 mg/kg
Hazard Type: Chemical
Animals per administration: 50
ROA: IP

Cyclophosphamide
Amount: 75-125 mg/kg
Hazard Type: Chemical
Animals per administration: 10
ROA: IP

Temozolomide
Amount: 50 mg/kg
Hazard Type: Chemical
Animals per administration: 50
ROA: IP

MDV3100
Amount: 10 mg/kg
Hazard Type: Chemical
Animals per administration: 32
ROA: Oral

CC-115
Amount: 50-100 mg/kg
Hazard Type: Chemical
Animals per administration: 32-80
ROA: IP

NU7441
Amount: 10 mg/kg
Hazard Type: Chemical
Animals per administration: 50
ROA: IP

Substance Immortalized Human Tumor Cell Lines

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Amount 1-10 million **Unit** Cells **Hazard Type** Biological

Frequency of Administration: one injection in each flank
Number of Animals per Administration: 1004

Route(s) of Administration

Route
IV
SC

Will the hazardous substances be brought into the animal facility for administration?

Is the agent/substance expected to be fully metabolized?

Are the metabolites known or expected to present any carcinogenic activity?

Are the metabolites known or be shed/excreted in urine/feces?

Does the material pose a hazard to laboratory employees (through direct contact with the hazard or direct or indirect co

Does the material pose a hazard to facility personnel (through direct contact with the hazard or direct or indirect contac

Is substance recombinant DNA? ○ ○ Yes ◉ ◉ **No**

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Substance Olaparib

Amount 100 **Unit** mg/kg **Hazard Type** Chemical

Frequency of Administration: twice daily M-F for four weeks
Number of Animals per Administration: 50

Route(s) of Administration

Route
IP

Will the hazardous substances be brought into the animal facility for administration?

Is the agent/substance expected to be fully metabolized?

Are the metabolites known or expected to present any carcinogenic activity?

Are the metabolites known or be shed/excreted in urine/feces?

Excretion Duration:

Does the material pose a hazard to laboratory employees (through direct contact with the hazard or direct or indirec

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Does the material pose a hazard to facility personnel (through direct contact with the hazard or direct or indirect contact

Substance NU7026

Amount 25 **Unit** mg/kg **Hazard Type** Chemical

Frequency of Administration: Twice daily M-F, for four weeks
Number of Animals per Administration: 50

Route(s) of Administration

Route
IP

Will the hazardous substances be brought into the animal facility for administration?

Is the agent/substance expected to be fully metabolized?

Are the metabolites known or expected to present any carcinogenic activity?

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Are the metabolites known or be shed/excreted in urine/feces?

Excretion Duration:

Does the material pose a hazard to laboratory employees (through direct contact with the hazard or direct or indirect co

Does the material pose a hazard to facility personnel (through direct contact with the hazard or direct or indirect contact

Substance Cyclophosphamide

Amount 75-125 **Unit** mg/kg **Hazard Type** Chemical

Frequency of Administration: 3x/week
Number of Animals per Administration: 10

Route(s) of Administration

Route
IP

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Will the hazardous substances be brought into the animal facility for administration?

Is the agent/substance expected to be fully metabolized?

Are the metabolites known or expected to present any carcinogenic activity?

Are the metabolites known or be shed/excreted in urine/feces?

Excretion Duration:

Does the material pose a hazard to laboratory employees (through direct contact with the hazard or direct or indirect co

Does the material pose a hazard to facility personnel (through direct contact with the hazard or direct or indirect contac

Substance Temozolomide

Amount 50 **Unit** mg/kg **Hazard Type** Chemical

Frequency of Administration: 1x/day for five days
Number of Animals per Administration: 50

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Route(s) of Administration

Route
IP

Will the hazardous substances be brought into the animal facility for administration?

Is the agent/substance expected to be fully metabolized?

Are the metabolites known or expected to present any carcinogenic activity?

Are the metabolites known or be shed/excreted in urine/feces?

Excretion Duration:

Does the material pose a hazard to laboratory employees (through direct contact with the hazard or direct or indirect co

Does the material pose a hazard to facility personnel (through direct contact with the hazard or direct or indirect contact

Substance MDV3100

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Amount 10 **Unit** mg/kg **Hazard Type** Chemical

Frequency of Administration: 1x daily M-F, for four weeks
Number of Animals per Administration: 32

Route(s) of Administration

Route
Oral

Will the hazardous substances be brought into the animal facility for administration?

Is the agent/substance expected to be fully metabolized?

Are the metabolites known or expected to present any carcinogenic activity?

Are the metabolites known or be shed/excreted in urine/feces?

Excretion Duration:

Does the material pose a hazard to laboratory employees (through direct contact with the hazard or direct or indirect co

Does the material pose a hazard to facility personnel (through direct contact with the **hazard or d**

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Substance CC-115

Amount 50-100 **Unit** mg/kg **Hazard Type** Chemical

Frequency of Administration: 2x day, M-F for four weeks
Number of Animals per Administration: 32-80

Route(s) of Administration

Route
IP

Will the hazardous substances be brought into the animal facility for administration?

Is the agent/substance expected to be fully metabolized?

Are the metabolites known or expected to present any carcinogenic activity?

Are the metabolites known or be shed/excreted in urine/feces?

Excretion Duration:

Does the material pose a hazard to laboratory employees (through direct contact with the hazard or direct or indirect co

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Does the material pose a hazard to facility personnel (through direct contact with the hazard or di

Substance NU7441

Amount 10 **Unit** mg/kg **Hazard Type** Chemical

Frequency of Administration: Twice daily M-F, for four weeks
Number of Animals per Administration: 50

Route(s) of Administration

Route
IP

Will the hazardous substances be brought into the animal facility for administration?

Is the agent/substance expected to be fully metabolized?

Are the metabolites known or expected to present any carcinogenic activity?

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Are the metabolites known or be shed/excreted in urine/feces?

Excretion Duration:

Does the material pose a hazard to laboratory employees (through direct contact with the hazard or direct or indirect co

Does the material pose a hazard to facility personnel (through direct contact with the hazard or direct or indirect contac

Immunizing Agent	Type	Adjuvant
There are no items to display		

Will ULAM Breeding Colony Management services be used? ○ Yes ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ Yes ○ No

Fee for Service Protocol
There are no items to display

Restraint/Device
There are no items to display

Tissue Type
Ear

Tail
Toe
Other

Immunizing Agent	Type	Adjuvant
There are no items to display		

Will ULAM Breeding Colony Management services be used? ○ Yes ○ No

Will post partum estrus for subsequent mating be intentionally utilized? ○ Yes ○ No

Fee for Service Protocol

There are no items to display

Restraint/Device

There are no items to display

Tissue Type
Ear
Tail
Toe

Other

Immunizing Agent	Type	Adjuvant
There are no items to display		

Will ULAM Breeding Colony Management services be used? Yes No

Will post partum estrus for subsequent mating be intentionally utilized? Yes No

Fee for Service Protocol

There are no items to display

Restraint/Device

There are no items to display

Tissue Type
Ear
Tail
Toe
Other

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Mouse

Procedure Details - Tumor Growth, Experimentally Induced

Indicate tumor endpoint (days, size, etc.).
The tumor endpoint will be when the tumor reaches 8x the starting volume or 90 days post treatment, which

Describe monitoring procedures by laboratory personnel for animals with tumors.
Tumors will be monitored by lab personnell at least two times a week using caliper measurements.

Are tumors expected to metastasize?	Yes	**No**
Are tumors expected to ulcerate?	**Yes**	No
Will all animals with ulcerated tumors be euthanized?	**Yes**	No

Justify why the animals with ulcerated tumors will not be euthanized.

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Procedure Description and Summary

Mouse
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Mouse

Describe in narrative form all experimental or instructional procedures in the order they will be performed on the animals, including procedures on anesthetized animals.

We will use adult immunocompromised mice (athymic nude, SCID, or NOD/SCID) for these studies. At the time of ordering or transfer from the breeding colony, the mice will be 3-5 weeks old and between 5-15 grams in weight.

After allowing the mice to acclimate to their new surroundings for approximately one week, we will establish prostate or sarcoma xenografts by injecting 1-10 million cultured in-vitro cancer cells subcutaneously into each flank of each mouse resulting in 2 tumors per mouse. During tumor cell injection, the mice will be anesthetized with isoflurane. When the tumors reach a size of 50-100mm3, we will begin a four week course of treatment with the combination of a DNA-PK inhibitor, anti-androgen MDV3100, other theraputic agents for prostate cancer (Olaparib or Temozolomide), and radiation. The Temozolomide treatment will be 1x/day for five days, not four weeks of treatment like the other theraputic agents described. The DNA-PK inhibitor, Olaparib and Temozolomide (TMZ) will be given will be given via an intraperitoneal injection. The anti-androgen MDV3100 will be given via oral gavage. The gavage will be done using a special gavage needle that will inject a predetermined volume of MDV3100 directly into the mouse's stomach. The radiation will be administered via external beam radiation therapy using an orthovoltage unit in the Medical Science Building I radiation core. The dose of radiation that will be given will be 2Gy/day for five days. Each 2Gy dose of radiation will last approximately 5 minutes per mouse, with each radiation session occurring 24 hours apart. Mice will be anesthetized prior to and during each radiation treatment. Tumor growth will be assessed at least twice weekly with caliper measurements. Animals will be euthanized when the tumor reaches 8x the starting volume or 90 days post treatment, whichever comes first. At the time of euthanization, the tumor will be harvested and frozen for further studies. Prior to euthanasia, mice will be anesthetized using isoflurane and blood will be drawn (0.5-1.0mL) by cardiac puncture. Immediately following the blood draw, the mice will be euthanized according to protocol.

There are five separate groups of planned experiments which are described below. Each of these experiments use the same procedures described in the above paragraph.

Experiment 1: Determination of the dose of DNA-PK inhibitor CC-115 to use in combination with radiation and anti-androgen therapy.
We will test varying doses of the DNA-PK inhibitor, CC-115, to use in combination with radiation. These studies will be performed for mice with prostate cancer xenografts and endpoints will include tumor control and weight loss (toxicity). The initial dose of CC-115 will be 50mg/kg, but may increase up to 100mg/kg in order to find the maximal dose. The maximal dose of CC-115 determined from these experiments will be used for all subsequent xenograft experiments. Mice will be euthanized one week after completion of the 4 week course of radiation therapy and CC-115 inhibitor.

Experiment 2: Effect of DNA-PK inhibitor CC-115 and radiation combined with either anti-androgen MDV3100 or Olaparib in VCaP prostate cancer cell xenografts.

Experiment 3: Effect of DNA-PK inhibitor CC-115 and radiation on tumor growth delay and tumor signaling in prostate cancer xenografts positive and negative for ETS gene fusions.

Experiment 4: Effect of DNA-PK inhibitor CC-115 alone and in combination with potential second-line therapeutics (Temozolomide, radiation, or Olaparib) on EWS-fusion-positive Ewing's sarcoma and EWS-fusion-negative control sarcoma xenograft models.

Experiment 5: Effect of DNA-PK inhibitor (NU7026, NU7441) and radiation on tumor growth delay and tumor signaling in prostate cancer xenografts.

Experiments 2-5 consist of treating various prostate cancer and sarcoma xenografts with the dose of a DNA-PK inhibitor established in Experiment #1 or from adequate doses from previously published literature, in combination with radiation therapy and other therapeutic agents. Endpoints will include tumor growth (described previously in the second paragraph, 8x starting volume or 90 days post treatment) and toxicity (weight loss). Experiments #1-5 all include using the same procedures, described in the second paragraph in this section.

In addition to experiments 1-5, we will be conducting a study investigating the effect of DNA-PK inhibitor (CC-115) and PARP inhibitor (Olaparib) on T-lineage acute lymphoblastic leukemia (T-ALL). Experiment #6 will consist of using female SCID or NOD/SCID mice aged 5-6 weeks. Prior to inoculation with T-ALL cells, mice will be warmed by infrared heat lamp and then inoculated with 2.5 - 10 million leukemia cells in a maximum volume of 100ul of PBS via tail vein injection. During the tail vein injection, mice will be anesthetized using ~2% Isoflurane and placed on their side. A small gauge needle is used for the injection, and back pressure is used to ensure that the needle is properly in the vessel. T-ALL cells are then injected into the tail vein. Post injection, gentle pressure is applied to the injection site until any bleeding has stopped. Mice will then be monitored for 5-10 minutes following injection procedure to make sure that bleeding has not resumed. Mice are then monitored every seven days for engraftment by withdrawing approximately 50ul of peripheral blood from the tail vein or saphenous vein and staining with anti-CD45 (leukocyte common antigen, Ly-5) antibodies. The proportion of human versus murine CD45+ cells will be calculated to assess overall leukemic burden. When the proportion of human CD45+ cells in the peripheral blood reaches an average of 1-5%, daily treatment with CC-115 in combination with Olaparib and/or cyclophosphamide will begin and continue for up to 4 weeks. We will be using the same dosages as previously stated in experiments #1-5 for CC-115 and Olaparib for this experiment, and will use 75-125mg/kg of cyclyphosphamide. When signs of morbidity (weight loss, lethargy, ruffled fur) appear or no more than 8 weeks following inoculation, mice will be euthanized according to protocol.

Will substances be administered to the animals in the course of experimentation that are reagent-grade rather than commercial pharmaceutical product? ○ Yes ◉ **No**

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Adverse Consequences

Mouse

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Mouse

Describe how you have refined experimental procedures to minimize pain and distress (e.g., early endpoints, use of analgesics, techniques that reduce stress, etc.).
In order to minimize pain and stress for the animals, during treatment the animals are monitored daily in order to recognize and correct potential problems early. The animals are also anesthetized during tumor cell injection (both subcutaneous and tail vein injections) as well as during irradiation treatment in order to alleviate the stress that may be caused by those procedures. If serious problems arise, the mice are euthanized at an earlier timepoint in order to reduce unnecessary suffering.

Describe expected adverse physical and/or physiological consequences, or adverse effects on well-being that the animals may experience as a result of the procedures.
All animals will be euthanized. We expect some weight loss (~10%) or less as a result of the DNA-PK inhibitors and radiation, which should resolve after the completion of treatment. DNA-PK inhibition alone has had no other adverse effects in other mouse xenograft studies.

For the tail vein injections, some peri-vascular irritation and a minimal amount of blood loss at the injection site might occur.

List severe complications that the animals may experience as a result of the procedures.
We do not expect severe complications, though anesthetic death is a possibility.

Describe how the consequences or events stated above will be monitored. Include criteria for premature euthanasia.
During the treatment period when most complications are likely to occur, the animals are monitored and weighed daily. Any animal experiencing greater than 15% weight loss for more than one day will be euthanized. The tumors do not generally ulcerate, but animals with ulcerated tumors will be euthanized. Also, the animals will be euthanized when their tumors reach the endpoint of 8x their starting volume, or 90 days post treatment. If blood loss is observed at the injection site on the tail, we will apply pressure until bleeding stops. In order to prevent perivascular irritation, we will infiltrate the tail around the vessel with saline. If there is evidence if tissue necrosis from administering the tail vein injections, the animals will be euthanized.

We will follow the Policy for End-Stage Illness and Humane Endpoints.

Will the Policy for End-Stage Illness and Humane Endpoints be followed? (•) **Yes** ( )

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No

Would analgesics be provided in human medicine for any of the procedures described?

Yes No

Will analgesics be provided to the animals for procedures? **Yes** No

PI: Feng, Felix Y
ID: PRO00003667
Status: Protocol Approved
Expiration Date: 3/19/2015

Use Categories and Justification

Mouse

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Mouse

Enter the total number of animals requested for each of the Use Categories for the next 3 years.

Use Category	Procedure Description	Examples	Ent Num o Anin

9	Painful or distressful experiments for which anesthetics, analgesics, tranquilizers are purposefully withheld because their use would adversely affect the experimental results	Trauma or burn on unanaesthetized animals	
8	Procedures conducted require the animals to feel pain in order to fulfill the aims of the study	Noxious stimuli, death as an endpoint, inflammation	
7	Procedures that cause distress, however no method is available which would alleviate these effects without interfering with the experimental results	Prolonged/chronic restraint, stress experiments, paralysis, prolonged food/water restriction	
6	Procedures that cause pain and/or distress, however anesthetics, analgesics, or tranquilizers are used to alleviate these effects	Major recovery surgical procedures (thoracotomy, craniotomy, laparotomy)	
5	Procedures that cause pain and/or distress which is alleviated by euthanasia	Debilitating tumor growth, toxicity tests, ascites production (Note: Animals are not permitted to spontaneously die, but will be euthanized when showing signs or morbidity)	
4	Procedures that cause pain and/or distress, however anesthesia is used and animals are not allowed to recover	Major surgical non-recovery procedures (organ/tissue removal, thoracotomy, perfusion under anesthesia, etc.)	
3	Procedures that cause some minor pain and/or distress but anesthetics and/or analgesics are used to alleviate these effects	Tail biopsy requiring anesthesia, implantation of peripheral catheters, subcutaneous implants, intracardiac injections	
2	Procedures that cause only slight or no pain or distress	Injections, breeding, tail biopsy (before 21 days of age in mice), non-debilitating tumor growth, anesthesia for chemical restraint, oral gavage, euthanasia	1148
1	No manipulations or procedures conducted	Observation without euthanasia at the end of the study	
		Total	1148

Justify the number of animals needed to achieve the scientific aim(s) of the project.

Experiment #1: Determination of the dose of DNA-PK inhibitor CC-115 to use in combination with radiation.

This experiment will require 10 mice per group, with 6 different doses of CC-115 assessed in combination with radiation therapy. Ten animals/group/endpoint are required for statistically meaningful data. Consequently a total of 60 animals will be needed for this experiment.

Experiment #2: Effect of DNA-PK inhibitor CC-115 and radiation combined with either anti-androgen MDV3100 or Olaparib in VCaP prostate cancer cell xenografts.

This experiment requires 10 animals per group for assessment of tumor growth delay, per our statistical calculations. In addition we will need another 6 animals per treatment group (3 animals whose endpoint will be once the first week of treatment is completed, and 3 whose endpoint will be once the third week of treatment has been completed). The tumors from these extra 6 animals per treatment group will be used for assessment of tumor signalling within the xenografts. Specifically, mice will be sacrificed for tumor harvest at these earlier timepoints so that signalling along the DNA-PK pathway (which should be blocked by CC-115) or the androgen receptor pathway (which should be blocked by MDV3100) or along the PARP-mediated pathway (which should be blocked by olaparib) can be assessed via immunoblot and immunofluoresecence approaches.

This results in 16 animals needed per group. There will be 9 treatment groups: control, radiation, CC-115 alone, MDV3100 alone, Olaparib alone, CC-115 + MDV3100, CC-115 + MDV3100 + radiation, CC-115 + Olaparib, and CC-115 + Olaparib + radiation.

This translates into 9 treatment groups x 16 animals per group = 144 animals needed.

Experiment #3: Effect of DNA-PK inhibitor CC-115 and radiation on tumor growth delay and tumor signaling in prostate cancer xenografts positive and negative for ETS gene fusions.

This experiment requires 10 animals per treatment group for assessment of tumor growth delay, as well as an additional 6 animals in each group for assessment of tumor signalling within the xenografts. There will be four different types of xenografts (derived from 2 prostate cancer cell lines, one an ETS-positive and one an ETS-negative, as well as PC3-ERG and PC3 LacZ xenografts). For each xenograft type, there will be four different treatment groups: control, radiation, DNA-PK inhibitor (CC-115), and radiation + DNA-PK inhibitor.

This translates into 4 xenograft models x 4 treatment groups x 16 animals per treatment group = 256 animals needed.

Experiment #4: Effect of DNA-PK inhibitor CC-115 alone and in combination with potential second-line therapeutics (Temozolomide, radiation, or Olaparib)on EWS-fusion-positive Ewing's sarcoma and EWS-fusion negative control sarcoma xenograft models.

This experiment requires 10 animals per treatment group for assessment of tumor growth delay, plus an additional 6 animals per group for assessment of tumor signalling within the xenograft. There will be two sets of xenograft models; one for RD-ES cells (Ewing's EWS-FLI1) and for A-204 cells (control sarcoma line). For each xenograft model, there will be nine treatment groups: control, CC-115, radiation, Temozolomide (TMZ), Olaparib, CC-115 + radiation, CC-115 + TMZ, CC-115 + Olaparib, CC-115 + Olaparib + radiation.

Thus, there will be 2 xenograft models x 9 treatment groups per model x 16 animals per treatment group = 288 animals.

Experiment #5: Effect of DNA-PK inhibitors NU7026 and NU7441 in combination with radiation on

tumor growth delay and tumor signaling in prostate cancer xenografts.

This experiment will use 2 cell lines to explore the effect of the DNA-PK inhibitors NU7026 and NU7441 in combination with radiation. Each drug will be used in two different xenograft models. There will be 10 animals in each treatment group to assess the tumor growth delay in addition to six animals per treatment group to assess tumor signalling within the xenograft. There will be four treatment groups: control, DNA-PK inhibitor, radiation, DNA-PK inhibitor + radiation.

This translates to 2 xenograft models x 4 treatment groups x 16 animals per treatment group x 2 DNA-PK inhibitors = 256 animals needed.

Experiment #6: Effect of DNA-PK inhibitor and PARP inhibitor on T-lineage acute lymphoblastic leukemia (T-ALL).

This experiment will use three cell lines to explore the effect of the DNA-PK inhibitor, CC-115, and the PARP inhibitor, Olaparib, on T-ALL. There will be 10 animals in each treatment group to assess the leukemia engraftment in addition to six animals per treatment group to assess signalling. There will be three treatment groups: control, DNA-PK inhibitor, PARP inhibitor.

This translates to 3 treatment groups x 3 cell lines x 16 animals per treatment group = 144 animals needed.

In summary, the total number of animals needed is 1148, as shown below:
Experiment #1: 60 animals
Experiment #2: 144 animals
Experiment #3: 256 animals
Experiment #4: 288 animals
Experiment #5: 256 animals
Experiment #6: 144 animals

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ID: PRO00003667
Status: Protocol Approved
Expiration Date: 3/19/2015

Principal Investigator's Assurance

Acknowledgment

I acknowledge responsibility for this project.

I have read the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training and certify that this project will be conducted in compliance with those

principles.

I assure that I will obtain UCUCA approval prior to significant changes in the protocol.

I assure that this project does not unnecessarily duplicate previous research or instructional projects.

I assure that students, staff, and faculty on the project are qualified or will be trained to conduct the project in a humane and scientific manner.

I assure that training records will be maintained for protocol-specific procedures for all students, staff, and faculty on the project.

By checking this box, I confirm the above statements and submit my electronic signature ☑

PI Last Notified: 3/6/2012 10:07 AM

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APPENDIX 2

Principal Investigator: Felix Feng
Protocol: PRO00003667
Protocol Title: DNAPK inhibition as a strategy for targeting ETS fusions in prostate cancer and ewing's sarcoma
Approval Period: 3/19/2012 - 3/19/2015
The University Committee on Use and Care of Animals (UCUCA) has reviewed your application to use vertebrate animals referenced below. This project has been approved. The proposed animal use procedures are in compliance with University guidelines, State and Federal regulations, and the standards of the "Guide for the Care and Use of Laboratory Animals."
When communicating with the UCUCA Office please refer to protocol PRO00003667.
The approval date is 3/19/2012 . The approval period is for three years from this date. However, the United States Department of Agriculture (USDA) requires an annual review of protocols to use animals. Therefore, each year of this protocol prior to the anniversary of its approval date, you will be notified via email to submit a short annual review. Your continued animal use approval is contingent upon the completion and return of this annual review.
You will also be notified prior to expiration so that your renewal application can be prepared, submitted and reviewed in a timely manner to avoid noncompliance. For any change to the study, an amendment must be submitted to the UCUCA for review and approval prior to the implementation of the proposed change.
The University's Animal Welfare Assurance Number with the NIH Office Of Laboratory Animal Welfare (OLAW) is A3114-01, and most recent date of accreditation by the Association For The Assessment And Accreditation Of Laboratory Animal Care International (AAALAC, Intl.) is November 6, 2009.
If you receive news media inquiries concerning any aspect of animal care or use in this project, please contact James Erickson, News and Information Services, 647-1842. If you have security concerns regarding the animals or animal facilities, contact Bill Bess, Director of Public Safety, 763-3434.
A formal approval letter will follow for each funding source listed on the protocol.
Sincerely,

Dr. Keith Cook, Ph.D.
Professor and Chairperson
University Committee on Use and Care of Animals
This is an eRAM system message. Please do not reply to this address.
For Questions:

1. For application or protocol specific questions, contact the UCUCA Office
   Phone (734) 763-8028
   Email ucuca.office@umich.edu
2. For technical issues, contact the ITS Help Desk
   Phone (734) 764-HELP (4-4357), option 4, between 8 AM and 5 PM Monday through Friday
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Vice Chair Membership

Fred Waldman, M.D., Ph.D.
Medical Director, Biospecimen Resource

July 8, 2011

Dr. Felix Feng, M.D.
The University of Michigan Medical Center
1500 East Medical Center Drive
Ann Arbor, MI 48109-5010

Dear Dr. Feng:

On behalf of the Radiation Therapy Oncology Group and the Translational Research Program, we would like to congratulate you on the approval of your Research Grant titled: *Assessment of ETS fusion and PTEN status on prostate cancer outcomes following radiotherapy, alone or in combination with hormonal therapy, in the definitive or salvage setting* (TRP#171). This is a resource-related research grant; it does not include a monetary award. The grant period is 8/1/11 to 7/30/14.

As Principal Investigator of RTOG TRP project #171, you are required to:

1. Agree that you have funding to conduct and complete the project.
2. Submit an interim progress report six (6) months after the start of the project and a final report 30 days after the end of the project period. The final report must be submitted by 8/31/2014. The report should be emailed to the TRP Administrator at RTOG-TRP@acr.org and will be distributed to TRP Committee and RTOG Statistical Unit for review.
3. Transmit all data to RTOG Headquarters that is developed as a result of this RTOG project;
4. Complete and sign a Material Transfer Agreement in order to receive RTOG biospecimen materials;
5. Return all unused biospecimen materials to the RTOG Biospecimen Resource;
6. Follow all publication guidelines on the RTOG website including RTOG review of all manuscripts prior to journal submission;
7. Agree that all publications, presentations and subsequent grant applications and/or awards resulting from RTOG projects must acknowledge the Translational Research Program of the RTOG by including a statement similar to: "This work was supported by the RTOG Translational Research Program, funded through grant U10CA21661 by the National Cancer Institute." One reprint of each publication produced as a result of this grant is to be sent to the RTOG publications department (RTOG_Publications@acr.org) and the RTOG TRP Administrator (RTOG-TRP@acr.org);
8. Provide an annual update on all publications, grant applications, and awards as a result of this RTOG supported Research Grant project.

Please print, sign and date two copies of this letter to indicate that you understand and agree to comply with these publication and reporting requirements. Keep one copy for your records and return one to the TRP Administrator by fax (215-928-0153) or email (RTOG-TRP@acr.org).

Again, congratulations on an excellent research grant with an important potential outcome.

Sincerely,

Adam P. Dicker, M.D., Ph.D.
Chairman Translational Research Committee

Walter J. Curran, Jr., M.D.
RTOG Group Chair

Felix Feng, M.D.
Principal Investigator

9/21/11
Date